

US EPA ARCHIVE DOCUMENT

**DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)**

1. **CHEMICAL:** Metolachlor PC Code No.: 108801

2. **TEST MATERIAL:** Metolachlor Technical Purity: 97%

3. **CITATION:**

Authors: Arthur E. Putt

Title: Metolachlor Technical - The Chronic Toxicity to *Daphnia magna* Under Flow-Through Conditions

Study Completion Date: September 22, 1995

Laboratory: Springborn Laboratories, Inc., Wareham, MA

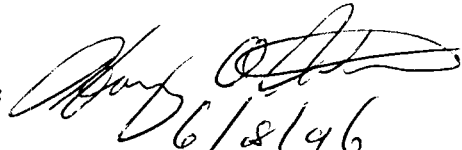
Sponsor: Ciba-Geigy Corporation, Greensboro, NC

Laboratory Report ID: 95-8-6061

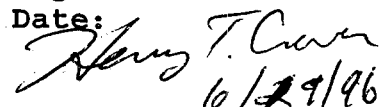
MRID No.: 438026-01

DP Barcode: D219942

4. **REVIEWED BY:** Harry A. Winnik
Biologist
EFED/EEB

Signature: 
Date: 6/28/96

5. **APPROVED BY:** Henry Craven
Supervisory Biologist
EFED/EEB

Signature:
Date:  6/29/96

6. **STUDY PARAMETERS:**

Age of Test Organism: ≤24 hours
Definitive Test Duration: 21 days
Study Method: Flow-Through
Type of Concentrations: Mean Measured

7. **CONCLUSIONS:** This study is scientifically sound but **does not fulfill** the guideline requirements (72-4(b)) for a freshwater invertebrate life-cycle test using *Daphnia magna*. The integrity of this study is questionable since measured concentrations were highly variable at all treatment levels throughout the study. The highest measured concentration was as much as 3.7 times higher than the lowest measured concentration within the same treatment level which exceeded the rejection rate criteria of 1.5 times. The study did not include raw growth data thus the growth data statistics could not be verified. This factor, plus the variability in the measured Metolachlor concentration, resulted in the classification of the study as supplemental. This study is not upgradable but does not need to be repeated at this time contingent upon the registrants acceptance of the NOEC LOEC and MATC values of 3.2, 6.9, and 4.7 ppm, respectively, based on growth and reproduction and the lowest measured concentration of each treatment level.



**DATA EVALUATION RECORD
AQUATIC INVERTEBRATE LIFE CYCLE TEST
GUIDELINE 72-4(B)**

1. **CHEMICAL:** Metolachlor PC Code No.: 108801

2. **TEST MATERIAL:** Metolachlor Technical Purity: 97%

3. **CITATION:**

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MRID No.: 438026-01

DP Barcode: D219942

4. **REVIEWED BY:** Rosemary Graham Mora, M.S., Aquatic Toxicologist, KBN Engineering and Applied Sciences, Inc.

Signature:

[Signature] for RBM

Date: 5/7/96

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature:

P. Kosalwat

Date: 5/7/96

5. **APPROVED BY:**

[Signature]

5/20/96

Signature:

William Robert for Henry Baker

Date: 6/5/96

6. **STUDY PARAMETERS:**

Age of Test Organism: ≤24 hours
Definitive Test Duration: 21 days
Study Method: Flow-Through
Type of Concentrations: Mean Measured

7. **CONCLUSIONS:** This study is not scientifically sound and does not fulfill the guideline requirements for a freshwater invertebrate life-cycle test using *Daphnia magna*. The integrity of this study is questionable since measured concentrations were highly variable at all treatment levels throughout the study. The highest measured concentration was as much as 3.7 times higher than the lowest measured concentration within the same treatment level. Based on mean measured concentrations, the MATC was between 5.9 and 12.0 ppm ai. The geometric mean MATC was 8.4 ppm ai.

Results Synopsis: Based on the lowest measured concentration of each treatment level, the following values will be considered valid for this study:

NOEC: 3.2 ppm LOEC: 6.9 ppm MATC: 4.7 ppm

8. ADEQUACY OF THE STUDY:

- A. **Classification:** supplemental
- B. **Rationale:** The acceptable values are based on the lowest measured concentration of each treatment level.
- C. **Repairability:** No

9. GUIDELINE DEVIATIONS: Since there is no EPA's SEP for a flow-through daphnid life-cycle test, the SEP for static renewal tests was used as a general guidance in this data validation.

- 1. The measured concentrations of test material in the exposure solutions were highly variable at all treatment levels throughout the study. The highest measured concentration was as much as 3.7 times higher than the lowest measured concentration within the same treatment level.
- 2. Individual growth data were not included in the report; therefore, the reviewer could not verify the author's conclusions.

10. SUBMISSION PURPOSE:

11. MATERIALS AND METHODS:

A. **Test Organisms/Acclimation:**

Guideline Criteria	Reported Information
<u>Species</u> <i>Daphnia magna</i>	<i>Daphnia magna</i>
<u>Source</u>	In-house culture

Guideline Criteria	Reported Information
<u>Parental Acclimation Conditions</u> Parental stock must be maintained separately from the brood culture in dilution water and under test conditions.	Parental acclimation conditions were not reported. Daphnid cultures were maintained under similar conditions as those used during the test.
<u>Parental Acclimation Period</u> At least 21 days.	Not reported.
<u>Age of Parental Stock</u> At least 10-12 days old at the beginning of the acclimation period.	Not reported.
<u>Food</u> Synthetic foods (trout chow), algae, or synthetic foods in combination with alfalfa yeast and algae.	<i>Ankistrodesmus falcatus</i>
<u>Food Concentration</u> 5 mg/l (dry wt.) of synthetic food or 10^8 cells/l of algae is recommended.	3.0 ml of algal suspension (4×10^7 cells/ml) two to three times daily
<u>Were daphnids in good health during acclimation period?</u>	Not reported.

B. Test System:

Guideline Criteria	Reported Information
<u>Test Water</u> Unpolluted well or spring that has been tested for contaminants, or appropriate reconstituted water (see ASTM for details).	Fortified well water which was filtered to remove potential organic contaminants.
<u>Water Temperature</u> 20°C \pm 2°C. Must not deviate from 20°C by more than 5°C for more than 48 hours.	Range: 20 \pm 0.7°C (daily measurements); 19-21°C (continuous measurement)

Guideline Criteria	Reported Information
<p>pH 7.6 to 8.0 is recommended. Must not deviate by more than one unit for more than 48 hours.</p>	7.9-8.3
<p>Total Hardness 160 to 180 mg/l as CaCO₃ is recommended.</p>	Mean total hardness of 170-180 mg/l as CaCO ₃
<p>Dissolved Oxygen <u>Renewal</u>: must not drop below 50% for more than 48 hours. <u>Flow-through</u>: ≥ 60% throughout test.</p>	Mean of ≥92% of saturation throughout the test
<p>Test Vessels or Compartments 1. <u>Material</u>: Glass, No. 316 stainless steel, or perfluorocarbon plastics 2. <u>Size</u>: 250 ml with 200 ml fill volume is preferred; 100 ml with 80 ml fill volume is acceptable.</p>	<p>1. Glass.</p> <p>2. 1.6-liter battery jars with a fill volume of approximately 1.4 l.</p>
<p>Covers <u>Renewal</u>: Test vessels should be covered with a glass plate. <u>Flow-through</u>: openings in test compartments should be covered with mesh nylon or stainless steel screen.</p>	Test vessels had screen-covered holes on each side.
<p>Type of Dilution System Must provide reproducible supply of toxicant. Intermittent flow proportional diluters or continuous flow serial diluters should be used.</p>	Intermittent-flow proportional diluter
<p>Flow Rate Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period.</p>	6 volume replacements/24 hours

Guideline Criteria	Reported Information
<u>Aeration</u> Dilution water should be vigorously aerated, but the test tanks should not be aerated.	Not reported. D.O. levels were $\geq 92\%$ of saturation throughout the test.
<u>Photoperiod</u> 16 hours light, 8 hours dark.	16 hours light, 8 hours dark
<u>Solvents</u> Not to exceed 0.5 ml/l for static tests or 0.1 ml/l for flow-through tests. Acceptable solvents are dimethyl formamide, triethylene glycol, methanol, acetone and ethanol.	Solvent: acetone Maximum conc.: 0.091 ml/l

C. Test Design:

Guideline Criteria	Reported Information
<u>Duration</u> 21 days	21 days
<u>Nominal Concentrations</u> Control(s) and at least 5 test concentrations; dilution factor not greater than 50%.	Dilution water control, solvent control (0.091 ml acetone/l) and 5 nominal concentrations: 2.5, 5.0, 10, 20, 40 mg ai/l
<u>Number of Test Organisms</u> 22 daphnids/level; 7 test chambers should contain 1 daphnid each, and 3 test chambers should contain 5 daphnids each.	10 daphnids/replicate; 4 replicates per treatment and control
Test organisms randomly or impartially assigned to test vessels?	Yes
<u>Renewal</u> Parent daphnids in all beakers must be transferred to containers with fresh test solution (< 4 hours old) three times each week (e.g. every Monday, Wednesday and Friday).	N/A

Guideline Criteria	Reported Information
Water Parameter Measurements 1. Dissolved oxygen must be measured at each concentration at least once a week. 2. pH, alkalinity, hardness, and conductance must be measured once a week in one test concentration and in one control. 3. Temperature should be monitored at least hourly throughout the test in one test chamber, and near the beginning, middle and end of the test in all test chambers.	1. D.O. and pH were measured weekly in each replicate and daily in one replicate of each treatment and control group. 2. Alkalinity, hardness, and conductivity were measured on Days 0, 7, 14, and 21 in one replicate of each treatment and control group. 3. Temperature was measured daily in one replicate of each treatment and control group, weekly in each replicate, and continuously in one control replicate.
Chemical Analysis Needed if chemical was volatile, insoluble, or known to absorb, if precipitate formed, if containers were not steel or glass, or if flow-through system was used.	Measured in two alternate replicates of each treatment and control group on Days 0, 7, 14, and 21.

Other Applicable Information: Throughout the exposure period, undissolved test material was observed in the diluter's mixing chamber and was removed twice daily. No undissolved test material was observed in diluter's chemical cells or exposure solutions during the test. To verify that no undissolved test material was present in the exposure solutions, additional samples were filtered prior to analysis.

12. REPORTED RESULTS:

A. General Results:

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes

Guideline Criteria	Reported Information
Control Mortality ≤ 30%	0% in dilution water control; 7.5% (3 out of 40) in solvent control
Did daphnids in each control produce at least 40 young after 21 days?	Yes
Were no ephippia produced in any of the controls?	Not reported.
Data Endpoints <ul style="list-style-type: none"> - Survival of first-generation daphnids, - Number of young produced per female, - Dry weight (required) and length (optional) of each first generation daphnid alive at the end of the test, - Observations of other effects or clinical signs. 	<ul style="list-style-type: none"> - Survival of first-generation daphnids, - Number of young produced, - Dry weight and length of surviving first-generation daphnids, - Clinical observations.
Raw data included?	Only survival and reproduction data were included. Raw data for length and dry weight were not included.

Effects Data:

Toxicant Concentration (ppm ai)		# dead daphnids/ total (%)	Mean Number Young per Female	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Mean Measured (SD)				
Control	<0.30	0/40 (0%)	92	4.9	1.00
Solvent Control	<0.30	3/40 (7.5%)	91	4.9	1.05
2.5	0.87 (0.44)	0/40 (0%)	90	4.8	0.92
5.0	1.8 (0.90)	2/40 (5%)	92	4.8	0.88

Toxicant Concentration (ppm ai)		# dead daphnids/ total (%)	Mean Number Young per Female	Mean Total Length (mm)	Mean Dry Weight (mg)
Nominal	Mean Measured (SD)				
10	2.9 (1.6)	2/40 (5%)	104	4.9	1.00
20	5.9 (3.1)	1/40 (2.5%)	106	4.9	1.10
40	12 (5.8)	0/40 (0%)	54 ^a	4.7 ^a	0.89 ^a

^a Significantly different from the pooled controls ($p \leq 0.05$).

Toxicity Observations: Throughout the study, no offspring were observed to be immobilized in any treatment or control group. Observations of adult daphnids revealed no treatment-related effects.

B. Statistical Results:

Most sensitive endpoint:

Endpoint	Method	NOEC (ppm ai)	LOEC (ppm ai)
Reproduction	Williams' Test	5.9	12
Weight	Williams' Test	5.9	12
Length	Williams' Test	5.9	12

13. VERIFICATION OF STATISTICAL RESULTS:

Most sensitive endpoint:

Endpoint	Method	NOEC (ppm ai)	LOEC (ppm ai)
Reproduction	ANOVA with Bonferroni t-test and Williams' test	5.9	12
Weight	Visual inspection	5.9	12
Length	Visual inspection	5.9	12

14. **REVIEWER'S COMMENTS:** Statistical analysis for growth could not be verified since individual growth data were not included in the report. By visual inspection, growth appeared to be affected only in the highest test concentration.

This study is scientifically sound but **does not** fulfill the guideline requirements for a daphnid life-cycle test. The integrity of this study is questionable since measured concentrations were highly variable at all treatment levels. With a high water solubility (760 ppm at 22°C as reported on page 14), it is not understandable why metolachlor precipitated out of solutions at the concentrations tested.

Prior to test initiation, the test system was reported to be maintaining test solutions at 22-32% of nominal concentrations; according to the author this "established that the diluter system was maintaining sufficient concentrations of test material in the exposure vessels to initiate the definitive study." During the in-life portion of the definitive test, the author reports that mean recoveries were consistently within the range of 29 to 37% of nominal fortified levels and that exposure samples which were filtered prior to analysis demonstrated recoveries consistent with the recoveries for the unfiltered samples. However, individual sample results demonstrated recoveries which ranged from 11 to 66% of nominal concentrations; the highest measured concentration was as much as 3.7 times higher than the lowest measured concentration within each treatment level (Tables 2 and 3, attached).

Several problems may be implied by the variability (e.g., a test material which is unstable under the study conditions, improperly prepared diluter stock solutions, or a diluter system which did not function properly during the study). The report did not include analysis of the diluter stock solutions; consequently, this factor cannot be dismissed. Results of the quality control samples indicate a problem with the diluter system (reportedly, the system function was consistent) or an effect of the solvent on the stability of the test material (note: it is unclear whether the solvent was used in the preparation of the quality control samples). Twenty of the 24 quality control samples demonstrated recoveries which ranged from 82.2 to 106.7% of nominal fortified concentrations (page 86, attached). The reviewer suggests that the cause of variability of the measured concentrations is unclear and the integrity of this study is questionable.

This study is classified as **Supplemental**. Based on the parameters of growth and reproduction and on the lowest measured concentration of each treatment level the NOEC, LOEC, and MATC values are 3.2, 6.9, and 4.7 ppm, respectively.

Table 2. Concentrations of metolachlor technical in replicate (unfiltered) exposure solutions during the 21-day chronic test with daphnids (*Daphnia magna*).

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L) ^a					Percent Nominal ^e
	Day 0 ^b	Day 7 ^c	Day 14 ^b	Day 21 ^c	Mean(SD) ^d	
Control	<0.22 <0.22	<0.23 <0.23	<0.26 <0.26	<0.30 <0.30	NA ^f	NA
Solvent Control	<0.22 <0.22	<0.23 <0.23	<0.26 <0.26	<0.30 <0.30	NA	NA
2.5	0.95 0.88	0.51 0.28	0.69 0.65	1.5 1.5	42% 0.87(0.44) 58%	35
5.0	1.4 ^g 1.7	1.1 0.92	1.7 1.5	3.2 3.3	44% 1.8 (0.90) 56%	37
10	2.5 2.1	1.6 1.5	2.3 2.1	5.6 5.3	49% 2.9 (1.6) 51%	29
20	5.2 5.1	3.1 3.3	4.7 4.5	11 11	47% 5.9 (3.1) 53%	30
40	11 12	7.1 6.6	10 9.5	22 21	46% 12 (5.8) 54%	31
QC ^h #1	38.6 (40.0) ^h	40.7 (40.0)	35.9 (40.0)	39.0 (40.0)		
QC #2	9.65 (10.0)	9.49 (10.0)	9.24 (10.0)	7.20 ⁱ (10.0)		
QC #3	2.95 (2.50) ⁱ	2.44 (2.50)	4.65 (2.50) ⁱ	2.34 (2.50)		

- ^a Measured concentrations were calculated based on the actual analytical (unrounded) data and not the rounded (two significant figures) data presented in this table. Mean measured concentrations were calculated based on days 0, 7, 14 and 21.
- ^b One water sample was collected from each replicate (A and B).
- ^c One water sample was collected from each replicate (C and D).
- ^d SD = standard deviation
- ^e Mean Percent Recovery = 39%
- ^f NA = Not applicable.
- ^g QC = Quality Control sample
- ^h Nominal fortified concentration for each QC sample is presented in parentheses.
- ⁱ Percent recovery for this QC sample was outside the standard range accepted by this laboratory (i.e., ± 3 standard deviations from the mean recovery established during the method validation/recovery study, Appendix V).

Table 3. Concentrations of metolachlor technical in replicate (filtered) exposure solutions during the 21-day chronic test with daphnids (*Daphnia magna*).

Nominal Concentration (mg A.I./L)	Measured Concentration (mg A.I./L) ^a				Mean(SD) ^d	Percent Nominal ^e
	Day 0 ^b	Day 7 ^c	Day 14 ^b	Day 21 ^c		
Control ^f	<0.22 <0.22	<0.23 <0.23	<0.26 <0.26	<0.30 <0.30	NA ^g	NA
Solvent Control ^f	<0.22 <0.22	<0.23 <0.23	<0.26 <0.26	<0.30 <0.30	NA	NA
2.5	0.67 0.64	— —	— —	— —	1.2 (0.66)	26
5.0	— —	— —	— —	— —		
10	1.9 1.6	— —	— —	— —	1.7(NA)	
20	— —	— —	3.2 2.7	9.3 9.5	6.2 (3.7)	31
40	8.1 9.6	5.7 6.8	7.6 9.9	19 17	10 (4.8)	26
QC ^h #1	32.9 (40.0) ⁱ	42.7 (40.0)	41.2 (40.0)	37.1 (40.0)		
QC #2	8.49 (10.0)	9.79 (10.0)	10.1 (10.0)	8.75 (10.0)		
QC #3	2.27 (2.50)	2.30 (2.50)	4.53 (2.50) ⁱ	2.44 (2.50)		

- ^a Measured concentrations were calculated based on the actual analytical (unrounded) data and not the rounded (two significant figures) data presented in this table. Mean measured concentrations were calculated based on days 0, 7, 14 and 21.
- ^b One water sample was collected from each replicate (A and B).
- ^c One water sample was collected from each replicate (C and D).
- ^d SD = standard deviation.
- ^e Mean percent recovery = 26%
- ^f Control solutions were unfiltered.
- ^g NA = Not applicable.
- ^h QC = Quality Control sample.
- ⁱ Nominal fortified concentration for each QC sample is presented in parentheses.
- ^j Percent recovery for this QC sample was outside the standard range accepted by this laboratory (i.e., ± 3 standard deviations from the mean recovery established during the method validation/recovery study, Appendix V).

SPRINGBORN LABORATORIES, INC.

Page 23

DATA SUMMARY TABLE FOR QUALITY CONTROLS

Sponsor: CIBA GEIGY
 Test Material: METOLACHLOR
 Project No.: 1781-0195-6484-130
 Test Type: 21 DAY LIFE CYCLE W/O. MAGMA
 Data Entered By: DL *NS*
 Date Program Run: 30-AUG-95

Sample ID	Nominal Concentration (MG/L)	Test Day	Analytical Result (MG/L)	Percent of Nominal
8-95-340C1	40.0	DAY 0	3.859E+01	96.5
7-95-350C2	10.0	DAY 0	9.649E+00	96.5
8-95-360C3	2.50	DAY 0	2.950E+00	118 (2)
8-95-750C1	40.0	DAY 0 (1)	3.287E+01	82.2
8-95-760C2	10.0	DAY 0 (1)	8.493E+00	84.9
8-95-770C3	2.50	DAY 0 (1)	2.276E+00	91.0
8-95-2450C1	40.0	DAY 7	4.071E+01	101.8
8-95-2460C2	10.0	DAY 7	9.494E+00	94.9
8-95-2470C3	2.50	DAY 7	2.439E+00	97.6
8-95-2500C1	40.0	DAY 7 (1)	4.268E+01	106.7
8-95-2510C2	10.0	DAY 7 (1)	9.792E+00	97.9
8-95-2520C3	2.50	DAY 7 (1)	2.301E+00	92.0
8-95-7490C1	40.0	DAY 14	3.592E+01	89.8
8-95-7500C2	10.0	DAY 14	9.239E+00	92.4
8-96-7510C3	2.50	DAY 14	① 4.033E+00 4.645E+00	186 (2)
8-95-7570C1	40.0	DAY 14 (1)	4.120E+01	103
8-95-7580C2	10.0	DAY 14 (1)	1.008E+01	101
8-95-7590C3	2.50	DAY 14 (1)	① 4.534E+00 4.155E+00	181 (2)
8-95-10570C1	40.0	DAY 21	3.896E+01	97.4
8-95-10580C2	10.0	DAY 21	7.203E+00	72.0 (2)
8-95-10590C3	2.50	DAY 21	2.336E+00	93.6
8-95-10650C1	40.0	DAY 21 (1)	3.710E+01	92.8
8-95-10660C2	10.0	DAY 21 (1)	8.746E+00	87.5
8-95-10670C3	2.50	DAY 21 (1)	2.443E+00	97.7

(1) These quality control samples were filtered through syringe filters prior to extraction.

(2) These quality control samples are outside the three standard deviation of 80.7 to 111. These values were not used in the mean.

① CEJAB 9/6/95

Metachlor: Number of Live Offspring

File: 43802601.you Transform: NO TRANSFORM

t-test of Solvent and Blank Controls

Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CTRL) MEAN = 840.5000 CALCULATED t VALUE = -1.0977
 GRP2 (BLANK CTRL) MEAN = 922.0000 DEGREES OF FREEDOM = 6
 DIFFERENCE IN MEANS = -81.5000

TABLE t VALUE (0.05 (2), 6) = 2.447 NO significant difference at alpha=0.05
 TABLE t VALUE (0.01 (2), 6) = 3.707 NO significant difference at alpha=0.01

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.876	6.776	10.696	6.776	1.876
OBSERVED	1	9	8	10	0

Calculated Chi-Square goodness of fit test statistic = 5.2285

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Shapiro - Wilk's test for normality

D = 213012.000

W = 0.979

Critical W (P = 0.05) (n = 28) = 0.924

Critical W (P = 0.01) (n = 28) = 0.896

Data PASS normality test at P=0.01 level. Continue analysis.

TITLE: Metachlor: Number of Live Offspring

FILE: 43802601.you

TRANSFORM: NO TRANSFORMATION

NUMBER OF GROUPS: 6

GRP	IDENTIFICATION	REP	VALUE	TRANS VALUE
1	GRPS 1&2 POOLED	1	942.0000	942.0000
1	GRPS 1&2 POOLED	2	747.0000	747.0000
1	GRPS 1&2 POOLED	3	711.0000	711.0000
1	GRPS 1&2 POOLED	4	962.0000	962.0000
1	GRPS 1&2 POOLED	5	998.0000	998.0000
1	GRPS 1&2 POOLED	6	911.0000	911.0000
1	GRPS 1&2 POOLED	7	828.0000	828.0000
1	GRPS 1&2 POOLED	8	951.0000	951.0000
2	0.87	1	845.0000	845.0000
2	0.87	2	1037.0000	1037.0000
2	0.87	3	889.0000	889.0000
2	0.87	4	826.0000	826.0000
3	1.8	1	740.0000	740.0000
3	1.8	2	1035.0000	1035.0000
3	1.8	3	874.0000	874.0000
3	1.8	4	899.0000	899.0000
4	2.9	1	978.0000	978.0000
4	2.9	2	1054.0000	1054.0000
4	2.9	3	1029.0000	1029.0000
4	2.9	4	951.0000	951.0000
5	5.9	1	956.0000	956.0000
5	5.9	2	1048.0000	1048.0000
5	5.9	3	1148.0000	1148.0000
5	5.9	4	1074.0000	1074.0000
6	12	1	478.0000	478.0000
6	12	2	515.0000	515.0000
6	12	3	706.0000	706.0000
6	12	4	470.0000	470.0000

Metachlor: Number of Live Offspring
 File: 43802601.you Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	GRPS 1&2 POOLED	8	711.000	998.000	881.250
2	0.87	4	826.000	1037.000	899.250
3	1.8	4	740.000	1035.000	887.000
4	2.9	4	951.000	1054.000	1003.000
5	5.9	4	956.000	1148.000	1056.500
6	12	4	470.000	706.000	542.250

Metachlor: Number of Live Offspring
 File: 43802601.you Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	GRPS 1&2 POOLED	11347.929	106.527	37.663	12.09
2	0.87	9129.583	95.549	47.774	10.63
3	1.8	14608.667	120.866	60.433	13.63
4	2.9	2202.000	46.925	23.463	4.68
5	5.9	6283.667	79.270	39.635	7.50
6	12	12301.583	110.913	55.456	20.45

Metachlor: Number of Live Offspring
 File: 43802601.you Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	643064.429	128612.886	13.283
Within (Error)	22	213012.000	9682.364	
Total	27	856076.429		

Critical F value = 2.66 (0.05,5,22)
 Since F > Critical F REJECT Ho: All equal

Metachlor: Number of Live Offspring
 File: 43802601.you Transform: NO TRANSFORMATION
 BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	881.250	881.250		
2	0.87	899.250	899.250	-0.299	
3	1.8	887.000	887.000	-0.095	
4	2.9	1003.000	1003.000	-2.021	
5	5.9	1056.500	1056.500	-2.908	
6	12	542.250	542.250	5.626	*

Bonferroni t table value = 2.51 (1 Tailed Value, P=0.05, df=22,5)

Metachlor: Number of Live Offspring
 File: 43802601.you Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

NUM OF Minimum Sig Diff % of DIFFERENCE

GROUP	IDENTIFICATION	REPS	(IN ORIG. UNITS)	CONTROL	FROM CONTROL
1	GRPS 1&2 POOLED	8			
2	0.87	4	151.142	17.2	-18.000
3	1.8	4	151.142	17.2	-5.750
4	2.9	4	151.142	17.2	-121.750
5	5.9	4	151.142	17.2	-175.250
6	12	4	151.142	17.2	339.000

Metachlor: Number of Live Offspring

File: 43802601.you

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 1 OF 2

GROUP	IDENTIFICATION	N	ORIGINAL MEAN	TRANSFORMED MEAN	ISOTONIZED MEAN
1	GRPS 1&2 POOLED	8	881.250	881.250	934.708
2	0.87	4	899.250	899.250	934.708
3	1.8	4	887.000	887.000	934.708
4	2.9	4	1003.000	1003.000	934.708
5	5.9	4	1056.500	1056.500	934.708
6	12	4	542.250	542.250	542.250

Metachlor: Number of Live Offspring

File: 43802601.you

Transform: NO TRANSFORMATION

WILLIAMS TEST (Isotonic regression model) TABLE 2 OF 2

IDENTIFICATION	ISOTONIZED MEAN	CALC. WILLIAMS	SIG P=.05	TABLE WILLIAMS	DEGREES OF FREEDOM
GRPS 1&2 POOLED	934.708				
0.87	934.708	0.887		1.72	k= 1, v=22
1.8	934.708	0.887		1.80	k= 2, v=22
2.9	934.708	0.887		1.83	k= 3, v=22
5.9	934.708	0.887		1.84	k= 4, v=22
12	542.250	5.626	*	1.85	k= 5, v=22

s = 98.399

Note: df used for table values are approximate when v > 20.